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## Component Proteins and Structure of Rice Ragged Stunt Virus

By K. HAGIWARA,<sup>1</sup>\* Y. MINOBE,<sup>1</sup> Y. NOZU,<sup>1</sup> H. HIBINO,<sup>2</sup> I. KIMURA<sup>1</sup>  
AND T. OMURA<sup>3</sup>

<sup>1</sup>National Institute of Agrobiological Resources, Tsukuba Science City, Ibaraki 305, Japan,

<sup>2</sup>International Rice Research Institute, P.O. Box 933, Manila, Philippines and

<sup>3</sup>National Agriculture Research Center, Tsukuba Science City, Ibaraki 305, Japan

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### SUMMARY

Rice ragged stunt virus (RRSV) particles consist of a polyhedral core particle approx. 50 nm in diameter to which are attached flat spikes about 20 nm wide and 10 nm high, giving a total diameter of about 70 nm. Polyacrylamide gel electrophoresis of disrupted particles of RRSV gave five major polypeptides with mol. wt. of 145000 (145K), 137K, 72K, 47K and 37K. Core particles prepared by suspending particles in 0.5 M-MgCl<sub>2</sub> contained the 145K, 137K, 72K proteins and relatively small amounts of the 37K protein, whereas the supernatant fluid recovered after centrifugation of the core particles contained the 47K and 37K proteins. The results suggest that the 145K, 137K and 72K proteins are core proteins and one or both of the 47K and 37K proteins are components of the spike. Immunoblotting experiments indicated that all the proteins are recognized as antigens, suggesting that RRSV particles are less stable than those of the phyto-reoviruses, rice dwarf and rice gall dwarf viruses.

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Rice ragged stunt virus (RRSV) causes a disease in rice and is transmitted in a persistent manner by brown planthoppers (Hibino *et al.*, 1977; Ling *et al.*, 1978; Boccardo & Milne, 1984). The genome of RRSV comprises 10 segments of double-stranded RNA (Omura *et al.*, 1983; Boccardo & Milne, 1984). No information has been reported on the structural proteins of RRSV presumably because it is difficult to obtain purified preparations. The numbers, sizes and location in the virus particle of plant reovirus structural proteins have been determined for wound tumour virus (Reddy & Macleod, 1976), rice dwarf virus (RDV) (Nakata *et al.*, 1978), rice gall dwarf virus (RGDV) (Omura *et al.*, 1985) and maize rough dwarf virus (Boccardo & Milne, 1975) by analysing intact and core particles obtained by several different methods. Proteins of core preparations of Fiji disease virus have also been described (Van der Lubbe *et al.*, 1979).

We report here the number and molecular weights of component proteins of RRSV particles and compare RRSV with other plant reoviruses. In addition, spikes of the particles were removed from the purified particle by MgCl<sub>2</sub> treatment and the location of some component proteins was deduced.

RRSV was purified by the method of Omura *et al.* (1983). The final pellets were resuspended in 0.1 M-histidine buffer containing 0.01 M-MgCl<sub>2</sub> adjusted to pH 7.0 with HCl (His-Mg) and stored at -70 °C.

To remove spikes from virus particles, purified preparations of RRSV suspended in 5 µl His-Mg were added to 2.5 µl MgCl<sub>2</sub> solutions at various concentrations. After incubation for 60 min at 30 °C, the preparations were ultracentrifuged for 30 min at 25 p.s.i. (130000 g) using a Beckman Air Fuge. The supernatant fraction, as well as the pellet resuspended in 7.5 µl His-Mg, were again centrifuged under the same conditions, and the resultant supernatant and pellet fractions were used for electron microscopy and electrophoresis.

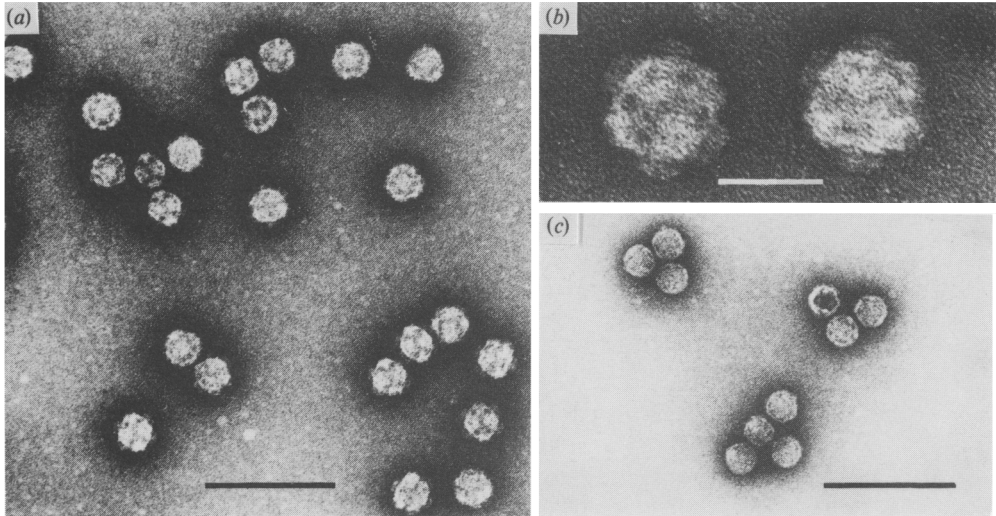


Fig. 1. Electron micrographs of RRSV particles fixed with glutaraldehyde and stained with 2% uranyl acetate. (a, b) Purified particles; bar markers represent 200 nm and 50 nm respectively. (c) Core particles; bar marker represents 200 nm.

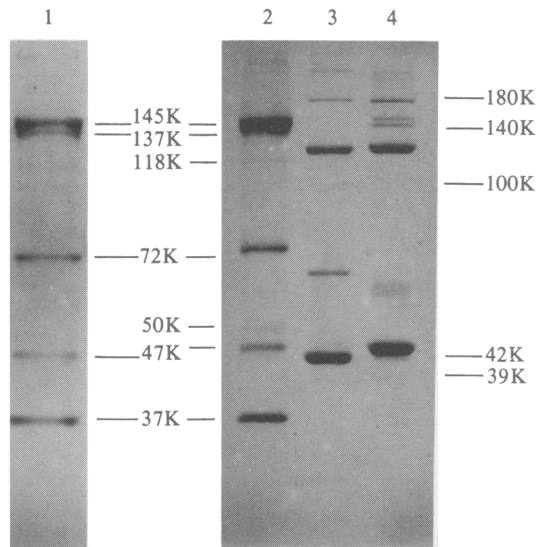


Fig. 2. SDS-polyacrylamide gel (10%) electrophoresis of proteins from particles of RRSV (lanes 1 and 2), RDV (lane 3) and RGDV (lane 4). Positions of RRSV species are shown.

For electron microscopy, preparations were fixed with 0.25% glutaraldehyde in 0.01 M-MgCl<sub>2</sub>, 0.03 M-triethanolamine pH 7.0 at 37°C for 15 min, negatively stained with uranyl acetate and examined in a Hitachi H-500 electron microscope. Purified preparations mostly consisted of polyhedral particles about 50 nm in diameter (core particles) surrounded by flat spikes about 20 nm wide and about 10 nm high (Fig. 1 a, b). The particle was approx. 70 nm from edge to edge of the spikes. These results agree with those reported by Kawano *et al.* (1983), which were considered to describe the complete particle of RRSV (Milne, 1980). So far, no particles with outer shells like those of Fijiviruses (Milne & Lovisolo, 1977) have been observed in preparations of RRSV, and the core particles with spikes seem to represent RRSV particles,

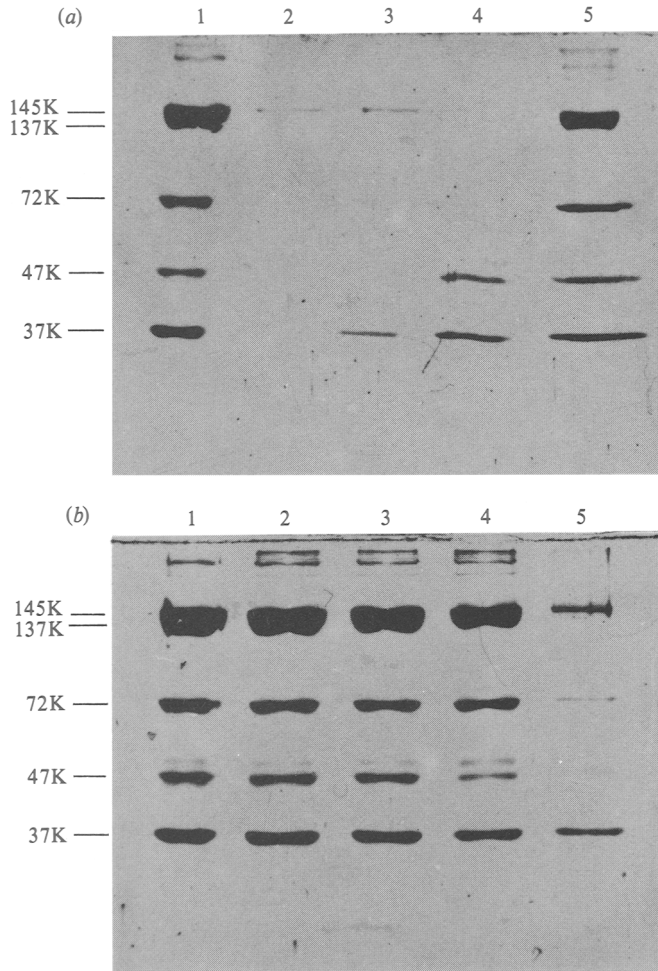


Fig. 3. Disruption of RRSV particles by  $MgCl_2$ . RRSV particles were treated with  $MgCl_2$  and fractionated by centrifugation into supernatant and pellet fractions. Protein in these fractions was electrophoresed in 10% gels. (a) Supernatant fraction. (b) Pellet fraction. Lane 1, SDS-dissociated whole virus; lanes 2 to 5, treatment with  $MgCl_2$  at 0.01 M (lane 2), 0.1 M (lane 3), 0.5 M (lane 4) and 2.0 M (lane 5).

although there is a possibility that the intact virion has an outer shell which is removed during purification or staining. Particles pelleted in  $MgCl_2$  solutions less concentrated than 0.1 M appeared to be almost the same as the untreated particles. The particles lost their spikes in 0.5 M- $MgCl_2$  (Fig. 1c) and no particles were pelleted in 2 M- $MgCl_2$ .

Electrophoresis of polypeptides, staining of the gels, scanning and molecular weight determination were performed according to the methods of Omura *et al.* (1985) except that protein was dissolved in 1% SDS and 1% 2-mercaptoethanol by heating a suspension for 1 min at 100 °C. Molecular weights of proteins were determined using SDS-PAGE Marker I [mol. wt. ( $\times 10^{-3}$ ) were 180, 140, 100, 42, 39] (Biochemicals, Tokyo, Japan). Proteins from RRSV particles were resolved into five major components by PAGE (Fig. 2a, b). The estimated mol. wt. were 145000 (145K), 137K, 72K, 47K and 37K. The relative amounts of each protein, measured by densitometry, were 60:12:7:21 for the 145K plus 137K, 72K, 47K and 37K proteins, respectively. Of the minor components, 118K and 50K were present in all preparations although always in trace amounts.

The molecular weight distribution of the major proteins of RRSV is unlike that of plant reoviruses (Nakata *et al.*, 1978; Reddy & Macleod, 1976; Nuss & Peterson, 1980; Omura *et al.*, 1985; Van der Lubbe *et al.*, 1979; Boccardo & Milne, 1975) (Fig. 2) and is perhaps a reflection of the lack of serological relationship between RRSV and the other plant reoviruses.

The amounts of the minor component proteins were too small to permit detailed study and in a study of the location of the protein components in particles, only the major proteins were considered. Core particles (Fig. 3) recovered as a pellet after treatment of particles with 0.5 M-MgCl<sub>2</sub> contained the 145K, 137K, 72K and 37K proteins although the relative amount of 37K in this fraction was less than in preparations from spiked core particles. The supernatant fraction contained the 47K and 37K proteins. All the protein species were found in the supernatant fraction when particles were treated with 2.0 M-MgCl<sub>2</sub>, which suggests that the particles had dissociated almost completely. Because the relative amounts of the 145K, 137K and 72K proteins in core preparations obtained by MgCl<sub>2</sub> treatment were almost the same as in spiked core preparations, we think that these three proteins are the components of core particles and that the 47K, and perhaps the 37K proteins which were removed from the core by 0.5 M-MgCl<sub>2</sub> treatment are the component proteins of the spike. However, the location of the 37K protein was not clear-cut, probably because of the labile nature of RRSV particles.

Polypeptides were transferred electrophoretically from polyacrylamide gels to nitrocellulose as described by Towbin *et al.* (1979), and reacted with rabbit antiserum to RRSV [titre 1/1280 in precipitin ring interface tests (Hibino & Kimura, 1982)] and horseradish peroxidase-conjugated sheep anti-rabbit IgG. RDV and RGDV proteins were used as controls. All the proteins of RRSV, RDV and RGDV were shown to have been transferred to nitrocellulose by amido black staining (data not shown) but only those of RRSV reacted with the antiserum. Furthermore, the antiserum reacted with all the component proteins of RRSV (data not shown). This contrasts with results from similar experiments with RDV and RGDV proteins (Minobe *et al.*, 1984; Omura *et al.*, 1985) in which it was mainly surface proteins that were recognized as antigens. However, antiserum to SDS-dissociated RDV particles did contain antibodies to all the component proteins of RDV particles (Matsuoka *et al.*, 1985). Probably the RRSV particles used as the immunogen dissociated during the immunization process but those of RDV and RGDV did not. This further suggests that RRSV particles are more labile than those of RDV and RGDV, both of which belong to the phyto-reovirus genus.

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